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Defining the Resistance Risk of the New Powdery Mildew Fungicide Quinoxyfen*

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Abstract: The new powdery mildew fungicide quinoxyfen belongs to the novel quinoline class of chemistry. Although its biochemical mode of action is unknown, quinoxyfen does not act in the same way as other cereal fungicides. It is a systemic protectant which inhibits the early stages of mildew infection on a wide range of crops, and provides season-long protection from a single early-season spray applied around GS 31.

The base-line sensitivity profile of quinoxyfen was defined for barley powdery mildew (*Erysiphe graminis* f.sp. *hordei*) from over 340 field isolates collected from different parts of the UK from 1991 onwards. Sensitivities ranged from $<0{\cdot}0001 \rightarrow 0{\cdot}16$ mg litre^{-1} with a mean of 0·003 mg litre^{-1}. Current work is extending the base-line sensitivity studies to wheat powdery mildew (*E. graminis* f.sp. *tritici*), and includes isolates from European trials, but so far this new data set has shown no differences from barley powdery mildew. Quinoxyfen-resistant mutants were generated in the laboratory, and some similar resistant strains were obtained from treated field crops. These laboratory and field strains were always defective, in some way, for sporulation and, curiously, all required the presence of quinoxyfen for survival in culture. Attempts to generate resistant mutants that sporulated normally were unsuccessful.

These studies suggested that the resistance risk for quinoxyfen is low. The recommended anti-resistance strategy accompanying introduction of quinoxyfen avoids seed treatments and late-season applications. Instead, a single early (GS 31) treatment using either pre-formulated mixtures or alternating with a fungicide with different mode of action is recommended. This strategy will be supported by continued monitoring of wheat and barley powdery mildew.

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1 INTRODUCTION

At present two major chemical classes are available for the control of mildew on wheat (caused by *Erysiphe graminis* DC f.sp. *tritici* Em. Marchal) and on barley (caused by *E. graminis* DC f.sp. *hordei* Em. Marchal); both of these chemical groups are sterol biosynthesis inhibitors and include cyproconazole, epoxiconazole and tebuconazole (azoles) which inhibit biosynthesis through C14 demethylation inhibition (DMIs),[1,2] while fenpropimorph, tridemorph and fenpropidin (morpholines and piperidines) work through the inhibition of Δ^{14}-reductase and $\Delta^8 \rightarrow \Delta^7$-isomerase.[3] Resistance to the DMIs is widespread in Europe, and has reduced the usefulness of azoles as powdery mildew specifics with clear positive cross-resistance between the various products.[4] Morpholine performance is now variable across Europe with a fall off in overall performance observed in the United Kingdom and France.[5,6] Although morpholines continue to have good curative activity, multiple applications are required to give long-term disease control leading to the highest yield benefits.[7] Other broad-spectrum compounds, such as the synthetic strobilurins, which affect mitochondrial electron transport, and anilinopyrimidines, which are amino acid biosynthesis inhibitors, are currently being developed and are being commercialised and these will help to provide solutions to the control of resistant mildew.[8,9]

The characteristics of quinoxyfen (DE-795), a new fungicide under development for the specific control of powdery mildews (Erysiphales), are described elsewhere.[10] The biochemical mode of action of quinoxyfen is under investigation; it is not a sterol demethylation inhibitor or a mitichondrial electron-transport inhibitor and does not have a mode of action corresponding with other currently commercialised fungicides or fungicides known to be under development (DowElanco, unpublished data).

In this paper the resistance profiling and resistance management recommendations for quinoxyfen are described.

2 MATERIALS AND METHODS

2.1 Baseline determination

In 1991, samples were collected from untreated winter barley sites throughout England and Scotland. From 1992 samples were collected from 35 × 50 m blocks of winter barley which were sprayed with 250 g ha^{-1} of a suspension concentrate formulation of quinoxyfen at second node growth stage.[11] From 1995, similar-sized wheat blocks were also treated and sampled. During 1996 samples of mildew were also collected from European trials locations.

Plants of susceptible varieties of barley (cv. Golden Promise) and of wheat were grown, five plants per pot, in a closed greenhouse and treated when three leaves were fully expanded with 250 g ha^{-1} of formulation of quinoxyfen. An equal number of plants was left unsprayed. Sampling of the mildew population was carried out by placing one pot of treated plants and one pot of untreated plants as pairs randomly through the treated crop. The treated and untreated sampling pots were placed in the crop at 21 and 42 days after treatment of the crop and collected one week later. The bait plants were incubated for a week before single pustule isolates were made. Leaf segments containing single pustules were cut from bait plants and allowed to sporulate. These single postule clones were transferred to fungicide-free leaf segments and maintained at 15°C and sub-cultured at 10-day intervals.

The bioassay method described in Reference 12 was used. Briefly, leaf segments of barley (cv. Halcyon) were floated on solutions of fungicide. Concentrations of quinoxyfen usually ranged from 0·0005 to 0·05 mg litre^{-1}. Inoculation of the segments with selected mildew isolates (using a settling tower) and their subsequent incubation and examination under a microscope enabled measurements of germ-tube growth and inhibition of appressorium formation to be made. Infection and growth were measured after 72 h incubation at 15°C, and sensitivity determined in terms of the dose needed to reduce growth by 50% (ED_{50}).

2.2 Cross-resistance

The bioassay method described in Section 2.1 was used. Cross-resistance patterns in barley powdery mildew to quinoxyfen, triadimenol, and fenpropidin were determined from both library and field isolate collections.

2.3 Mutagenesis

Ten-day-old barley seedlings (cv. Halycon) were inoculated with conidia of a wild-type barley mildew isolate (23D5), which had never been exposed to fungicides. After 24 h the first leaves were removed and their cut ends placed in a solution (2 ml) containing 2-aminobenzimidazole (100 mg litre^{-1}) and *N*-nitrosoguanidine (NTG; 25 mg litre^{-1}). Uptake was allowed to continue for four days and the segments were then transferred to water agar (5 g litre^{-1}) for sporulation to occur. Clones were established on fungicide-free leaf material from both the NTG-treated and untreated sources. The number of pustules that developed from both inoculated sources provided a measure of the percentage kill caused by *N*-nitrosoguanidine. These clones were transferred with a paint brush to leaves of the same barley cultivar

sprayed to run-off with a suspension concentrate formulation of quinoxyfen (0·16 mg AI litre^{-1}). Growth was assessed after seven days.

2.4 Conidia production

Seedlings infected with either IW 240 ('resistant' isolate, collected from the field in 1993) or 23D5 (wild-type) were shaken after seven days to remove existing conidia. Forty-eight hours later, 10 leaves with (as far as possible) similar levels of infection, were carefully removed from each plant and placed in ethanol + water (1 + 1 by volume; 2·5 ml). Tubes were shaken and conidia counted using a haemocytometer. Conidia production was expressed as the number produced per leaf.

Conidia produced after exposure to NTG for four days were inoculated onto quinoxyfen-treated or untreated leaf segments, and pustule numbers counted seven days later. This infection provided the inoculum source for the same testing procedure up to the fourth generation.

3 RESULTS AND DISCUSSION

3.1 Baseline determination

The overall sensitivity distribution of barley isolates to quinoxyfen is shown in Fig. 1. The median peak is in the 0·001–0·005 mg litre^{-1} range (0·003 by measurement of the median of the concentration range). There was no significant difference between isolates collected from the field on either treated or untreated plants.

The data from 1996 European studies are shown in Fig. 2.

The data sets for barley and wheat in Figs 1 and 2 show similar sensitivity distributions. No isolates from treated crops gave results outside the assay range shown.

3.2 Cross-resistance

No cross-resistance between triadimenol and quinoxyfen was observed (Table 1). Isolate DH14 is ethirimol-resistant,[12] suggesting no cross-resistance between

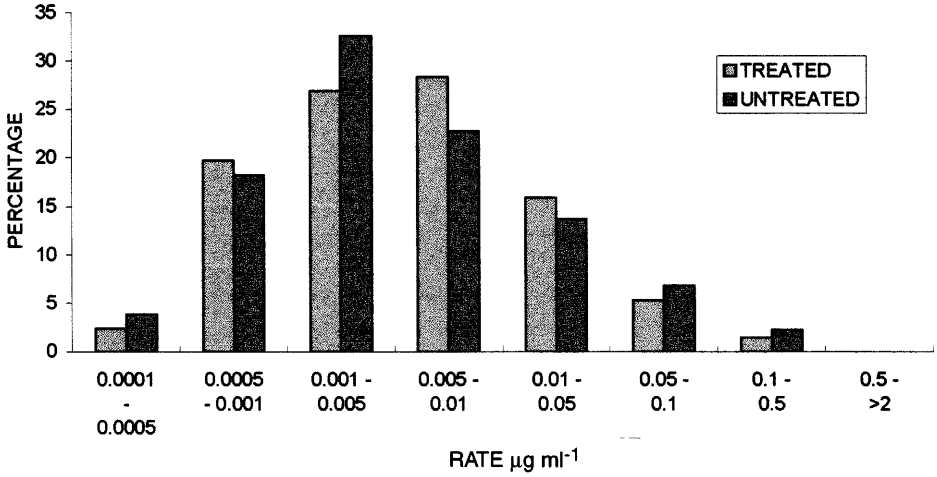

Fig. 1. Quinoxyfen resistance: base line sensitivity 1991–1994.

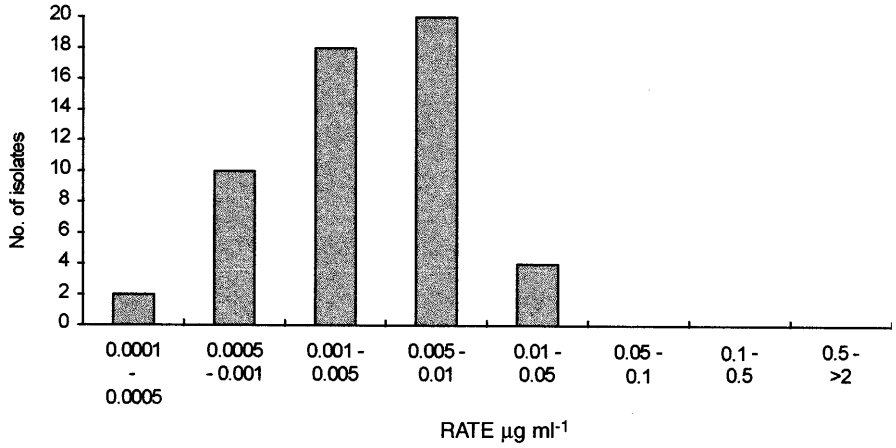

Fig. 2. Quinoxyfen: Base sensitivity of wheat powdery mildew isolates. France 1996.

TABLE 1
Cross-Resistance Patterns in Barley Powdery Mildew to Quinoxyfen and Triadimenol[a] (Library Isolates)

Strain[b]	*Year of isolation*	*Sensitivity (ED_{50} mg litre^{-1})*	
		Triadimenol	*Quinoxyfen*
DH14(1)	1976	0·004	0·001
DH14(2)	1976	0·008	0·008
23D5	1973	0·007	0·06
JB212	1982	0·459	0·0005
CSB5	1989	0·51	0·009
CSB22	1989	0·62	0·002
BSB18	1989	0·70	0·081
CSB9	1989	0·70	0·003
JB1753	1988	1·68	0·005
CSB2	1989	1·72	>5·0
JB1931	1989	1·85	0·071
JB1641(1)	1987	2·06	0·004
JB1641(2)	1987	7·8	0·015
BUSB22	1989	4·3	0·008
BUSB6	1989	4·8	0·1
BUSB20	1989	6·8	<0·0005
L32	1989	6·0	0·0008
BUSB2	1989	7·4	0·09
1704*	1987	1·78	0·01
1705*	1988	0·72	0·0008
E10*	1986	2·24	0·002

[a] Using this laboratory test, differences must be greater than 10-fold to achieve significance at the 95% level (validated for triadimenol).
[b] Most tests were carried out in 1990; those marked * in 1991.

quinoxyfen and this 2-aminopyrimidine mildewicide. With one exception (CSB2), all the 'library' isolates were quinoxyfen-sensitive with ED_{50} values varying from >0·0005 to 0·1 mg litre^{-1}. Unfortunately, no further work was carried out on CSB2 as it was soon lost from the culture collection. In this respect, CSB2 resembled mutants subsequently produced in the laboratory by mutagenesis with NTG (e.g. NTG1), or collected from the field in quinoxyfen-treated crops.

Although fenpropidin and fenpropimorph belong to different chemical classes there was very little variation in sensitivity to library isolates and this was supported by data from field isolates (Table 2).

TABLE 2
Cross-Resistance Patterns in Barley Powdery Mildew to Quinoxyfen and Fenpropidin[a] (Field-Collected Isolates)

Strain[b]	*Year of isolation*	*Sensitivity (ED_{50} ; mg litre^{-1})*	
		Fenpropidin	*Quinoxyfen*
23D5*(1)	1973	0·12	0·001
23D5 (2)		0·08	0·004
IW26	1991	0·35	0·02
IW31	1991	0·09	<0·0005
IW32	1991	0·35	0·01
IW35	1991	0·13	0·005
IW45	1991	0·32	0·005
IW69	1991	0·03	0·001
IW81	1991	0·002	0·001
IW82	1991	0·01	0·005

[a] Using this laboratory test, differences must be greater than 10-fold to achieve significance at the 95% level (validated for fenpropidin). Tests carried out in 1991.
[b] * Library 'wild-type' reference.

3.3 Mutagenesis

Exposure of the wild-type isolate 23D5 to NTG, followed by selection on quinoxyfen-treated leaves generated mutants which were able to grow on these treated leaves. All these mutants seemed to require the presence of quinoxyfen for normal infection and growth; in the absence of the fungicide, mutants grew poorly and could not be maintained on untreated leaves (Table 3).

Studies of these mutants and the field 'resistant' isolates revealed that conidia production was reduced, largely as a result of poor conidiophore production. The wild-type 23D5 typically produced more than three times as many conidia as the field 'resistant' isolate. Similar observations were made with laboratory-induced mutants. Back-cross attempts to produce viable mutants were unsuccessful.

TABLE 3
Viability of Wild-Type (23D5) and Laboratory Mutant (NTG1) in the Presence or Absence of Quinoxyfen (0·16 mg litre^{-1})

	Fungicide treatment	*Number of pustules per leaf after each generation*			
		1	*2*	*3*	*4*
Wild-type (23D5)	No quinoxyfen	115	84	73	127
	+ quinoxyfen	4	0	0	0
NTG1	No quinoxyfen	17	6	8	1
	+ quinoxyfen	63	85	98	82

It was concluded that these isolates were of low fitness and consequently the potential to cause epidemics is limited.

4 CONCLUSIONS

The baseline sensitivity (ED_{50}) of over 340 field isolates of *E. graminis* f.sp. *hordei* to the fungicide quinoxyfen ranged from $< 0{\cdot}0001$ to $0{\cdot}016$ mg litre^{-1}, with a median of $0{\cdot}003$ mg litre^{-1}. These isolates were all collected in the UK from 1991 onwards, while the compound was under development and not available commercially. During 1996 field isolates of *E. graminis* f.sp. *tritici* collected in France produced a similar distribution and no 'resistant' strains were observed. This broad range in baseline sensitivity was similar to that observed for other mildew fungicides.[13,14]

Barley mildew isolates with ED_{50} values of $>0{\cdot}16$ mg litre^{-1} were isolated with a low frequency from treated crops, but could only be maintained on quinoxyfen-treated leaves. *E. graminis* f.sp. *hordei* strains 'resistant' phenotypes could be generated in the laboratory after exposure to the chemical mutagen NTG. These mutants were also dependent on quinoxyfen for their continued maintenance in the culture collection. Detailed studies of both these mutants and the field 'resistant' isolated revealed that conidia production was reduced, largely as a result of poor conidiophore production. It was concluded that these isolates were of low fitness and consequently the potential of such isolates to cause epidemics is limited.

No cross-resistance was found between quinoxyfen and a representative azole (triadimenol) or between quinoxyfen and fenpropidin.

Based on the available data, a resistance management strategy has been drawn up:

1. Restriction of exposure of the pathogen by not developing seed treatments and not recommending foliar use after the first awns' visible growth stage (BBCH49);
2. development of pre-formulated mixtures and tank-mix recommendations with other fungicide groups of different modes of action; and
3. alternation of quinoxyfen with fungicides of different modes of action when mildew control outside the quinoxyfen window is needed.

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